Contribution to the life-history of Sequoia sempervirens.

WALTER ROBERT SHAW.

WITH PLATE XXIV.

Sequoia sempervirens Endlicher and *S. gigantea* Decaisne are the only living representatives of a once larger and very widely distributed genus of the Coniferæ. Each species is limited to a narrow natural distribution in California. We have no account of the development of the sexual generation (prothallium), our knowledge of the reproduction in this genus being limited to accounts of the development of the flowers[1] and the germination of the seed[2] of *S. sempervirens*. The arboretum of Leland Stanford Junior University contains a large number of young fruiting trees of the latter species. It also lies within the zone of distribution of the species, there being one tree one hundred and forty feet high on the university grounds. At the suggestion of Dr. Douglas H. Campbell a study of the development of the macrosporangia (ovules) and the prothallia by microtome methods was begun in November, 1891, and carried on under his direction. The publication of the results was several times delayed in hope of being able to make them more complete.

The material for study was collected from young trees in the arboretum of Stanford University during the season of 1891–92. Collections were made in December, 1891 (not dated), and from January 9 to July 5, 1892, at intervals of three to seven days. The young flowers were split longitudinally, and the sporophylls were removed from the older flowers. The specimens were fixed in 1 per cent. chromic acid for eighteen hours, washed in water, and transferred gradually to 90 per cent. alcohol. For sectioning the alcoholic material was stained *in toto* in Czokor's alum-cochineal and imbedded, through the medium of turpentine, in paraffin. The sections were cut on a Minot microtome, stained on the slide with a solution of Bismarck brown in 70 per cent. alcohol, and mounted in Canada balsam.

[1] Strasburger, Die Angiospermen und die Gymnospermen 85. Jena, 1879.
[2] Strasburger, Die Coniferen und die Gnetaceen 327. Jena, 1872.

The female flower.

About the first of December, 1891, it was found that the macrosporangiate (female) flowers on larger trees were more advanced in development than those on the smallest trees which bore flowers. The sporophylls (cone scales) of these flowers are closely arranged spirally on an axis which is at this time about 4^{mm} in length, and are surrounded by scale leaves which are borne lower down on the same axis. Each macrosporophyll consists of a shorter basal portion perpendicular to the axis of the flower, and a longer terminal portion, closely appressed and parallel to the axis of the flower (compare figs. 6 and 7). On the ventral (upper) side of the basal portion is a transverse row of macrosporangia one to eight or ten in number, most numerous on the middle sporophylls of each flower. The middle sporangium on each sporophyll has its axis nearly parallel with, but slightly inclined toward, the floral axis. In the terminal portion of the sporophyll there is a resin-duct (*r* in figs. 6 and 7) along the dorsal (lower or outer) side of the single fibrovascular bundle. The abrupt bend between the basal and terminal parts of the sporophyll is somewhat thickened transversely in all directions. The sporangia are cylindrical and about as long as broad (figs. 1 and 2), and the integument reaches to a level with the flat top of the sporangium.

Just as the flowers had closed in February, Mr. B. M. Davis collected a hermaphrodite flower. In this flower the upper sporophylls are macrosporangiate and like those of the female flowers, and five of the lower sporophylls are microsporangiate (male) and, with the exception of the upper one which is in form like a female sporophyll, are similar to those of the regular male flowers. The relative positions of the two kinds of sporophylls is the same as the relative positions which the two kinds of flowers occupy on the branches of the tree; the female flowers are formed on the terminal shoot and neighboring twigs of each fertile branch, and the male flowers on the lateral twigs of the same branches. And correspondingly the lower branches of the tree bear more male than female flowers.

Early in January the microsporangiate flowers shed their pollen and the macrosporangiate flowers open to receive it. The opening of the flowers, male as well as female, consists in a separation of the sporophylls by intercalary growth of

the floral axis accompanied by an elongation of the basal portion of each sporophyll. The growth of the axis is greatest between the lowest sporophyll and the upper scale leaves, so that the flower is carried out of its envelope. In the macrosporangiate flower the basal part of the axis is negatively geotropic at this time and the flower assumes a more or less upright position. Open flowers were collected for about a month during which time the integument grows out beyond the sporangium forming a vestibule into which a thick fluid substance is excreted. In this the pollen grains are caught and held. About the time when the flowers open, the transverse thickening in the bend between the basal and terminal portions of the sporophyll begins to increase. This thickening develops in all directions nearly at right angles to the basal portion of the sporophyll and by it the flower is closed. This growth involves also that part of the base of the sporophyll which bears the sporangia and by it the sporangia are inclined toward the floral axis (figs. 6 and 7). When the flowers close, early in February, the middle sporangia on each sporophyll are about half way turned toward the axis, and about the first of March the micropyles are directed toward the floral axis. In the thickened part of the sporophyll secondary resin-ducts are developed and in the base of the sporophyll fibrovascular bundles are formed which end beneath the sporangia.

The cones continue to grow until about the first of June, at which time they are 21–24^{mm} in length and 15–17^{mm} in thickness. The cones open by shrinkage of the fleshy, obconical, middle portion of the sporophyll, which takes place at the end of the summer, in September, or later in the same year.

The macrosporangia.

In the young flowers the macrosporangia are circular in cross section and the integument reaches up to a level with the flat top of the sporangium. The sporangium is about as long as broad. The integument consists of the inner and outer epidermis and two layers of hypodermal cells. About the time the flowers open, January 1st, the integument begins to exceed the sporangium in length, and when the flower closes, about a month later, the integument is about twice as long as the sporangium. The micropyle then begins to close by radial elongation of the integument epidermal cells about it. In this way the pollen grains on the flat circular top of

the sporangium become enclosed in a subconical cavity, the micropyle. About the time when the micropyle begins to close, the hypodermal tissue of two opposite sides of the integument begins to grow in a radial direction to form the wings of the seed. In some cases the thickening of the integument occurs on three or four sides, in the directions of least resistance, but only two wings are developed. About the middle of February, when the micropyle has closed, the hypodermal cells in that part of the integument which surrounds it develop thick pitted walls which appear to be lignified (fig. 5, *e*). Up to this time the sporangium has grown slowly and it now begins to elongate by growth of the chalazal portion (fig. 3). The seed reaches its full length and width in June, when it is about 5^{mm} wide by 6^{mm} long.

In December the sporangium is cylindrical in form and about as long as broad, and surrounded by the integument which reaches about to the same level. Within the epidermis are five to seven central longitudinal rows of cells surrounded by one or two layers of smaller cells which are also arranged in longitudinal rows. Each of the central rows of cells appears to have originated in a single cell immediately beneath the epidermis, and the rows extend from the apex of the sporangium nearly to the chalaza, in which no regular arrangement of the cells can usually be traced. In the earliest stage observed the central rows consisted of two or three cells (fig. 1). Later stages show several cells in each row, of which the innermost, larger and longitudinally elongated, with a large nucleus, is a sporogenous cell, and the others are tapetal cells which are often somewhat flattened in shape with smaller and lenticular nuclei (fig. 2). During January the sporangium elongates slightly and there come to be about six tapetal cells between each sporogenous cell and the epidermis at the micropyle (fig. 3, *b*). About the first of February, after pollination has been effected, the growth of the sporangium becomes limited to the region between the sporogenous cells and the chalaza. The tapetum anterior to and alongside the sporogenous cells undergoes no farther growth and the cell walls of the anterior tapetum become somewhat thick and firm. By the basal growth of the sporangium the sporogenous cells come to occupy a position relatively near the apex of the sporangium (figs. 4, *c*, and 5, *e*). About the middle of March the cells immediately about the base of the

somewhat enlarged sporogenous cell or cells begin to weaken and disorganize (fig. 4, *d*), and each sporogenous cell divides twice to produce four macrospores (fig. 5, *f*). The first division is transverse and the second, which follows before the first wall develops to any thickness, is transverse in the lower cell and either transverse, oblique, or longitudinal in the upper cell.

The female prothallium.

A number of spores begin at once to develop female prothallia. They increase in length and grow toward the chalaza at the expense of the cells which lie in their paths, and the growing end of each becomes gradually larger. After about three weeks, April 8th, sacs are found with two nuclei (fig. 8, *h*). Already one sac is considerably larger than the others. The elongation of the sporangium continues and the embryo-sacs grow rapidly in length. Usually the largest sac in a sporangium grows down through the center of the sporangium, and the smaller ones grow obliquely or spirally downward alongside the larger one. By the middle of April there are eight or sixteen nuclei in the largest sac, all located near the lower or growing end of the sac, and the rest of the sac contains little protoplasm. One or even more of the smaller sacs may contain as many nuclei as the larger sac and they also are collected near the lower end (fig. 9). As the sacs elongate, the nuclei become more numerous and are distributed in a peripheral layer of protoplasm which line the whole length of each sac. About the end of May the longest sac reaches to the chalaza and the lower two-thirds or three-fourths becomes thicker. Usually the smaller sacs are confined to the upper third or quarter of the sporangium where they become tangled and surround the upper part of the principal sac. This upper part of the principal sac becomes atrophied and does not develop tissue. The formation of the cellular prothallium in the sac was not observed but it takes place about the first of June. When this occurs all or nearly all of the tissue of the sporangium has been absorbed except the epidermis and those cells which were anterior to the sporogenous cells. The upper part of the sporangium containing the secondary sacs and the "suspensor" of the primary sac becomes shriveled and bent. The cells of the prothallium usually show an arrangement in radial rows but do

not always. The cells in the upper end are as a rule larger than those in the lower end.

The date of maturation of the archegonia seems to vary as much as a month. The archegonia are numerous and usually arranged radially in the upper half or third of the prothallium, sometimes distributed to the upper end and sometimes not. They are, then, as a rule lateral. Only a few preparations showed the archegonia. In these the archegonia were nearly as long as half the transverse diameter of the prothallium and each consisted of a small neck cell and a large egg-mother cell (fig. 10, *s*, *t*). The farther development of the archegonia remains to be studied.

By July 5th the central part of the upper half of the prothallium contains several intertwined tubular suspensors each with an eight- or twelve-celled embryo on the lower end (figs. 11 and 12). The origin of the proembryos (suspensors with embryos) could not be traced in the sections. From prothallia macerated for a few hours in 10 per cent. caustic potash suspensors were obtained by dissection. They are unicellular and each contains a rather large nucleus near the middle, or the lower end. In nearly every case the wall of the upper end is ruptured as if by the penetration of a pollen-tube.

The pollen-tube.

At the time the pollen is shed in January each grain contains two apparent cells, a larger vegetative cell with a large nucleus, and a smaller, lenticular, parietal cell with a smaller nucleus. The germination of the pollen begins soon after the middle of February, and by the end of the third week in that month the pollen-tube reaches across the flat top of the sporangium and begins to grow down between the sporangium and integument. The vegetative nucleus passes into the tube and is usually to be found between the middle and the growing end of the tube. There is more variation in the rate of development of the pollen-tubes than of the principal embryo-sacs. During March the tube may reach half or two-thirds the length of the small sporangium and quite as often as not it branches, one branch growing on downward and the other taking any direction between the sporangium and integument, or penetrating the epidermis of the sporangium. About the time when the tube enters the sporangium the antheridial cell in the microspore enlarges and its nucleus divides (fig. 8, *i*, Apr. 8th). The two daughter nuclei, which

are smaller than the vegetative nucleus, move together into the tube. In a number of preparations these two nuclei were seen a short distance behind the vegetative nucleus near the place where the tube penetrates the sporangium wall. After entering the sporangium the tube passes obliquely downward and enlarges considerably. It soon becomes impossible to distinguish it in sections from the numerous windings of the several variously developed embryo-sacs with which it intertwines; and so it was not traced to the mature female prothallium. A study of the sectioned material is often made still more confusing by the fact that one or more of the secondary embryo-sacs with their free nuclei sometimes escape from the sporangia and grow around, up or down inside the integument. These are however larger than the pollen-tubes. An attempt was made to isolate the older pollen-tubes by macerating the sporangia in 5 per cent. and 10 per cent. caustic potash solutions but without success. It was found that this method showed clearly the course of the tubes before entering the sporangia but not farther. Some sectioned specimens show with little doubt that the pollen-tube usually or at least frequently grows down alongside the female prothallium, but as some of the secondary embryo-sacs with free nuclei often do the same thing nothing more definite was learned.

In some cases several of the embryo-sacs develop tissue, and again the single large prothallium may appear as several in sections by reason of constrictions produced by other immature sacs or by pollen-tubes.

The peculiarity of the pollen-tubes is that they do not penetrate the wall of the sporangium in the immediate neighborhood of the micropyle but at lateral points in the upper fifth of the sporangium. In this respect, and in the distinctly branched form which the tube develops,[3] Sequoia bears at least a remote resemblance to some of the so-called chalazogamous angiosperms.

With respect to the numerous archegonia and their irregular distribution in the prothallia we have in Sequoia an exceptionally generalized type of conifer. The division of each sporogenous cell into four macrospores, and the prolonged development of the secondary embryo-sacs, are characters as primitive as any we find among the Coniferæ. The abortion of the upper fourth or fifth of the principal embryo-sac into a

[3] S. Nawaschin, Botanisches Centralblatt **63:** 355. 1895.

suspensor-like structure the author does not know to occur in any other of the Gymnospermæ. The knowledge obtained of the development of the sporangia and prothallia indicates that the Taxodineæ have been very properly, if *Sequoia* is a fair type of the family, considered as a most primitive group of modern Coniferæ.

Stanford University, California.

Explanation of Plate XXIV.

Fig. 1. Longitudinal section of sporangium with integument. × 266. About Dec. 1, 1891.

Fig. 2. Longitudinal section through sporangium with integument, showing three sporogenous cells. × 266. About Dec. 10, 1891.

Fig. 3. *a*, longitudinal section through sporangium and integument. × 36. *b*, sporangium in same section showing three sporogenous cells. × 176. Feb. 16, 1892.

Fig. 4. *c*, longitudinal section through sporangium and integument. × 36. *d*, sporangium of same section showing one sporogenous cell. × 178. March 14, 1892.

Fig. 5. *e*, longitudinal section through sporangium with integument. × 34. *f*, sporangium of same section showing eight macrospores just formed from two sporogenous cells. × 180. March 14, 1892.

Fig. 6. Longitudinal section through sporophyll showing position of sporangium; the resin-duct, *r*, does not appear in its full length in this section. × 21. January 17, 1892.

Fig. 7. Median longitudinal section through sporophyll; *r*, resin-duct. × 21. January 17, 1892.

Fig. 8. *h*, longitudinal section through sporangium with male prothallia (pollen-tubes) and an embryo sac with two nuclei; the broken line indicates the course of a tube as followed in a different section, the tube being entirely outside of the sporangium; a nucleus at the point where the tube branches, and two nuclei in the antheridial cell; also two nuclei in the larger embryo sac. × 90. *i*, pollen spore of the same tube, drawn from two sections, showing the divided antheridial cell. × 154. April 8, 1892.

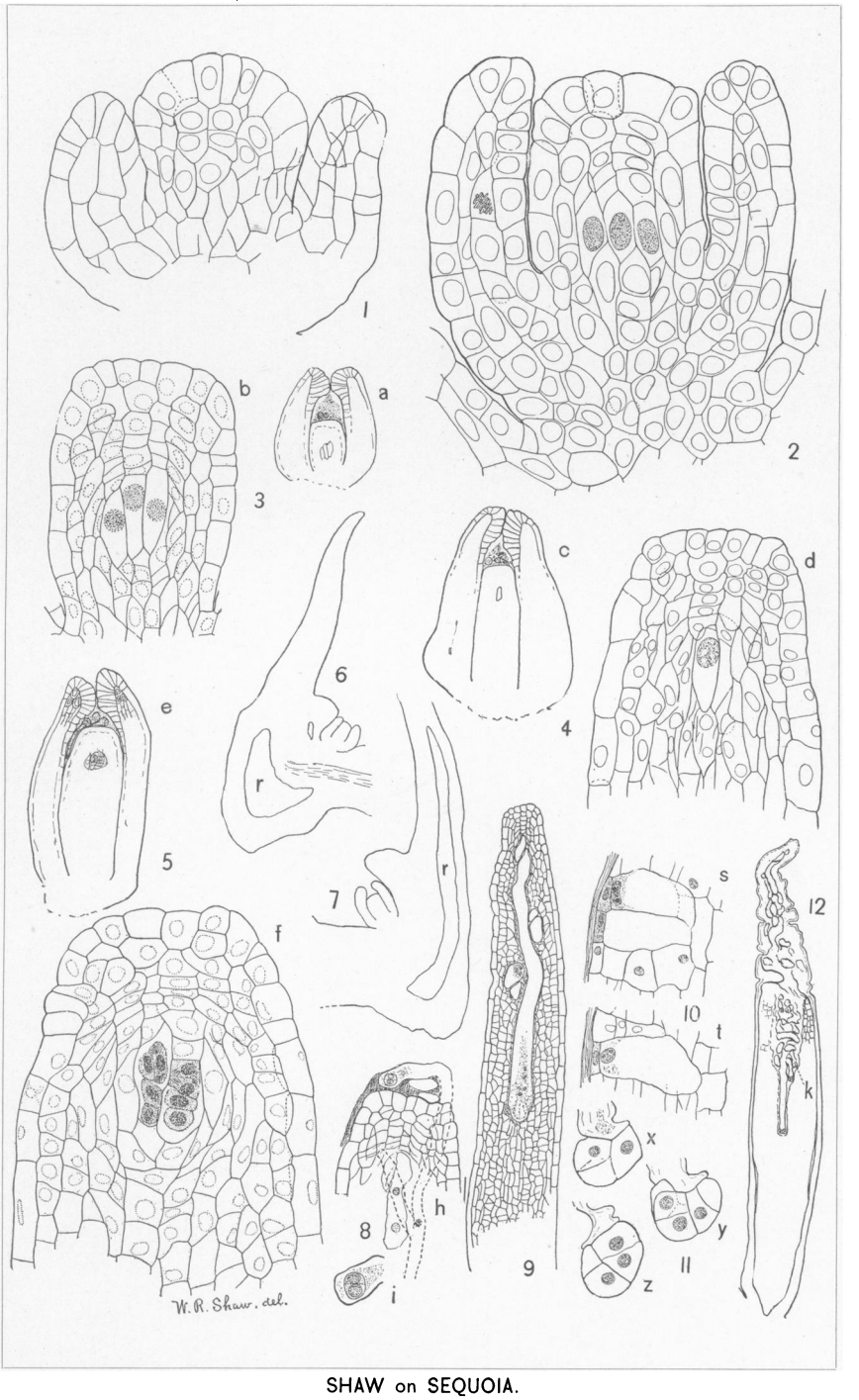
Fig. 9. Longitudinal section through a sporangium containing an embryo-sac with sixteen nuclei in the lower end, of which five appear in the section; two smaller sacs which wind about the larger one are cut in oblique section, one of the smaller sacs contains about sixteen nuclei, of which three appear in the section. × 41. April 15, 1892.

Fig. 10. Archegonia from a longitudinal section of a prothallium; *s*, from about one-fourth the length of the prothallium from the anterior end; *t*, about three-eighths from the anterior end. × 110. About June 21, 1892.

Fig. 11. Section of an embryo of eight cells from a longitudinal section of a prothallium; *x*, *y*, *z*, indicate the order of the sections. × 172. July 5, 1892.

Fig. 12. Longitudinal section through prothallium enclosed in sporangium of which the upper one-fourth is shrunken and occupied by abortive embryo-sacs; the upper half of the prothallium contains suspensors with embryos; *k* is the embryo section of fig. 11 *x*. × 15. July 5, 1892.

All drawings sketched with an Abbé camera lucida.



SHAW on SEQUOIA.